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SENSITIVITY PROFILE OF CEFTRIAXONE–EDTA–SULBACTAM AGAINST GRAM-NEGATIVE ISOLATES FROM ICU OF A TERTIARY CARE HOSPITAL

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ABSTRACT

Introduction: Antimicrobial resistance (AMR) is an escalating global concern, especially in intensive care unit (ICU) settings, where multidrug-resistant gram-negative bacteria are commonly encountered. ICU conditions including invasive procedures, prolonged hospital stay, and heavy antibiotic exposure predispose to infections by organisms producing extended-spectrum β-lactamases (ESBLs) or metallo- -lactamases (MBLs), limiting the effectiveness of conventional antibiotics. **Aim:** This study was designed to evaluate the in-vitro sensitivity of the combination Ceftriaxone–Sulbactam–EDTA (CSE) against gram-negative isolates obtained from ICU patients, and to compare its antimicrobial efficacy with that of plain Ceftriaxone and Ceftriaxone–EDTA–Clavulanic acid (CEC). **Methods:** A cross-sectional study was conducted involving 30 non-duplicate gram-negative isolates from clinical specimens (urine, sputum, pus, blood, and sterile body fluids) collected in the ICU of a tertiary care hospital. Isolates were cultured on Blood agar and MacConkey agar and identified using standard biochemical tests. Antimicrobial susceptibility was assessed via the Kirby–Bauer disk diffusion method, following the guidelines of the Clinical Laboratory Standards Institute (CLSI M100, 2021). Three antibiotic regimens Ceftriaxone alone, CEC, and CSE were tested and zone diameters interpreted accordingly. **Results:** Out of the 30 gram-negative isolates, CSE demonstrated a sensitivity rate of 96.7%, markedly higher than CEC (56.6%) and Ceftriaxone alone (40%). When analyzed by organism type, CSE exhibited 100% susceptibility against isolates of Escherichia coli, Klebsiella pneumoniae, Citrobacter spp., Acinetobacter spp., Proteus spp. and Enterobacter spp., while among Pseudomonas spp. the sensitivity was 75%. **Conclusion:** The high in-vitro sensitivity of CSE across diverse gram-negative isolates suggests that CSE may be a promising therapeutic alternative for multidrug-resistant infections in ICU settings, potentially reducing dependency on last-resort agents. However, further studies including molecular resistance profiling, minimum inhibitory concentration (MIC) determination, and clinical outcome evaluation are warranted before recommending widespread adoption.

KEYWORDS : Antimicrobial resistance (AMR), Ceftriaxone–Sulbactam–EDTA (CSE), Gram-Negative Bacteria, Intensive care unit.

INTRODUCTION:

Antimicrobial resistance (AMR) among Gram-negative bacteria has emerged as a formidable global health challenge, especially in hospital intensive care units (ICUs), where critically ill patients are at high risk due to invasive procedures, prolonged hospitalization, frequent antibiotic use, and immunosuppression. ICU environments often become breeding grounds for multidrug-resistant (MDR) organisms. Among these, extended-spectrum β-lactamase (ESBL) and metallo-β-lactamase (MBL) producing pathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter* spp., *Pseudomonas aeruginosa*, *Proteus* spp., *Enterobacter* spp., *Citrobacter* spp. are frequently implicated in severe nosocomial infections.

These organisms pose a major therapeutic challenge, as they exhibit resistance against many conventional β-lactam antibiotics, including third-generation cephalosporins, and increasingly even carbapenems. Overreliance on last-resort drugs like carbapenems or colistin has led to growing reports of carbapenem-resistance and concerns about toxicity, cost, and stewardship [1]. In response to this crisis, there is an urgent need for effective, affordable, and safer alternatives preferably carbapenem-sparing agents. One such promising candidate is the fixed-dose combination of Ceftriaxone, Sulbactam, and EDTA (disodium edetate) collectively referred to as "CSE." The rationale behind this combination is rooted in synergy: ceftriaxone provides broad-spectrum β-lactam activity; sulbactam acts as a β-lactamase inhibitor and has documented activity against certain non-fermenters (e.g., *Acinetobacter* spp.); while EDTA, a metal-chelating agent may inhibit MBL enzymes, disrupt bacterial biofilms, and increase membrane permeability to enhance antibiotic penetration [2].

Previous in-vitro and observational studies have reported encouraging results for CSE. For example, a multicenter Indian study involving over 3,000 Gram-negative clinical isolates found high overall susceptibility of ESBL- and MBL-producing bacteria to CSE, underscoring its potential as a carbapenem-sparing alternative. Another study [3] testing 179 MDR Gram-negative isolates (both ESBL and MBL producers) in a tertiary care setting demonstrated substantial in-vitro susceptibility, especially among ESBL-producers [4].

Nevertheless, data remain limited in many regions and clinical contexts particularly in ICU settings of tertiary care hospitals. There remains a need to evaluate local susceptibility patterns to ensure that CSE remains effective and to guide empirical therapy. Against this background, the present study was undertaken to assess the in-vitro sensitivity of CSE among Gram-negative isolates obtained from ICU patients over a defined period, and to compare its performance with that of ceftriaxone alone and a comparator combination of ceftriaxone–EDTA–Clavulanic acid (CEC).

We hypothesized that CSE would demonstrate superior in-vitro activity compared to the other regimens, thereby

validating its potential as an effective empirical option in ICU settings burdened by MDR Gram-negative infections.

MATERIALS AND METHODS:

Study Design and Setting

This cross-sectional observational study was conducted over a two-month period in the post graduate dept. of microbiology in Acharya shri Chander college of medical sciences & hospital (ASCOMS & H) . The study population included patients admitted in various ICUs, from whom clinical specimens were collected for culture and sensitivity testing. Only one isolate per patient was included (non-duplicate isolates) to avoid bias.

Specimen Collection And Bacterial Identification

Clinical specimens included urine, sputum, pus, blood, and other sterile fluids — as relevant to patient presentation. Specimens were cultured on standard media: Blood agar and MacConkey agar. Bacterial colonies were processed for Gram-negative identification using conventional biochemical tests including triple sugar iron (TSI), citrate utilization, indole test, urease, oxidase, motility assays, and other relevant tests as per standard manuals (e.g., Mackie & McCartney, Koneman's Atlas).

Antibiotic Susceptibility Testing

Antibiotic sensitivity was evaluated using the Kirby–Bauer disk diffusion method. Three antibiotic regimens were tested:

- Ceftriaxone alone.
- Ceftriaxone–EDTA–Clavulanic acid (CEC).
- Ceftriaxone–Sulbactam–EDTA (CSE).

Commercially prepared discs (or in-house prepared as per standard protocols) were used. Zone diameters were measured and interpreted according to the guidelines provided by the Clinical Laboratory Standards Institute (CLSI M100, 2021 edition).

Only non-repetitive Gram-negative isolates recovered during the study period were included. Data were collated and sensitivity percentages for each regimen were calculated overall and, where possible, stratified by organism type.

Data Analysis:

Descriptive statistics were used to calculate the proportion of susceptible isolates for each antibiotic regimen. Sensitivity rates were reported as percentages. No advanced inferential statistics were performed given the limited sample size and observational design.

RESULTS:

Table 1: Antimicrobial Susceptibility of Gram-Negative Isolates (ICU specimens)

Organism (Gram-negative)	Number of isolates (n)	% Susceptible to CSE (Ceftriaxone–Sulbactam–EDTA)
Escherichia coli	(of 30)	100 %
Klebsiella pneumoniae	(of 30)	100 %
Citrobacter spp.	(of 30)	100 %
Acinetobacter spp.	(of 30)	100 %
Proteus spp.	(of 30)	100 %
Enterobacter spp.	(of 30)	100 %
Pseudomonas spp.	(of 30)	75 %
Overall (all isolates)	30	96.7 %

As shown in Table 1, among the 30 non-duplicate gram-negative isolates from ICU clinical specimens, the combination of Ceftriaxone–Sulbactam–EDTA (CSE) exhibited very high in-vitro activity. For six of the bacterial groups — *Escherichia coli, Klebsiella pneumoniae, Citrobacter* spp., *Acinetobacter* spp., *Proteus* spp., *and Enterobacter spp.* — 100 % of isolates tested were susceptible to CSE. In the case of *Pseudomonas* spp., the susceptibility rate was 75 %. Thus, across the diverse array of gram-negative pathogens, CSE demonstrated broadly consistent effectiveness, with only a modest reduction in susceptibility against *Pseudomonas* spp.

Table2 : Comparative Susceptibility — CSE, CEC, and Ceftriaxone (All Isolates)

Antibiotic regimen	% Susceptible
Ceftriaxone–Sulbactam–EDTA (CSE)	96.7 %
Ceftriaxone–EDTA–Clavulanic acid (CEC)	56.6 %
Ceftriaxone (alone)	40.0 %

Table 2 presents a regimen-wise comparison of in-vitro susceptibility across all 30 gram-negative isolates. The CSE regimen showed the highest overall sensitivity rate at 96.7 %, indicating nearly all isolates were inhibited. In stark contrast, the Ceftriaxone–EDTA–Clavulanic acid (CEC) combination achieved a sensitivity rate of 56.6 %, and plain Ceftriaxone alone was effective in only 40.0 % of cases. These results illustrate that the triple-component CSE regimen substantially outperforms both the simpler β-lactam/β-lactamase inhibitor combination (CEC) and the standalone β-lactam (Ceftriaxone) in this isolate collection.

DISCUSSION

In this study, the CSE combination demonstrated markedly higher in-vitro susceptibility among Gram-negative isolates compared with both ceftriaxone alone and the CEC regimen. The high overall sensitivity suggests that the triple-component formulation effectively counters the resistance mechanisms prevalent among ICU pathogens — including ESBL and MBL production, biofilm formation, and other defense strategies.

The performance of CSE in our findings aligns with prior larger studies. For example, the multicenter evaluation of 3,150 Gram-negative clinical isolates reported robust activity of CSE against ESBL- and MBL-producing bacteria from various species including *E. coli*, *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa* [5]. Likewise, observational studies from tertiary-care centers in India have described CSE as a promising "carbapenem-sparing" agent, with susceptibility rates ranging from moderate to high depending on strain and resistance mechanism [6].

Mechanistically, the enhanced activity of CSE likely arises from the complementary roles of its components. Sulbactam's -lactamase inhibition plus possible intrinsic activity against certain non-fermenters (e.g., *Acinetobacter*) expands the spectrum beyond what ceftriaxone alone could achieve. Concurrently, EDTA may chelate divalent metal ions (e.g., zinc) required for MBL activity, thereby inhibiting MBLs which represent a key resistance mechanism in many carbapenem-resistant isolates. Furthermore, EDTA may disrupt bacterial outer-membrane integrity and biofilms, thereby facilitating greater antibiotic penetration and potency [7].

The potential clinical implications are significant. In resource-limited settings or institutions with high prevalence of ESBL/MBL producers, CSE could serve as an empirical therapy reducing reliance on carbapenems or colistin, preserving these last-line agents, and possibly mitigating the risk of further resistance development. The inclusion of CSE in routine susceptibility panels (as recommended in some studies) could help guide therapy more appropriately.

However, and importantly the promising in-vitro results must be interpreted with caution. Several studies report variable efficacy of CSE, particularly against MBL-producing organisms. For instance, in one analysis of 179 MDR isolates (ESBL + MBL), only 68.2% of ESBL producers and 39.4% of MBL producers were susceptible to CSE [8]. This indicates that

while CSE may offer good coverage against many ESBL producers, its reliability against MBL producers especially highly resistant or carbapenem-resistant strains may be limited.

Furthermore, the translation from in-vitro susceptibility to in-vivo clinical efficacy is not straightforward. Clinical outcomes depend on multiple factors beyond susceptibility: pharmacokinetics and pharmacodynamics (PK/PD) of the drug combination in critically ill patients; the ability to achieve effective tissue and serum concentrations; patient comorbidities; presence of biofilms in vivo; immune status; and potential toxicity or side effects. Indeed, a randomized controlled clinical trial of CSE versus standard "best alternative treatment" (BAT) for complicated urinary tract infections caused by MBL-producing Enterobacterales failed to meet non-inferiority criteria [10]. This underscores the risk in over-relying on in-vitro data without robust clinical evidence.

Additionally, frequent and indiscriminate use of any "resistance-breaker" combination including CSE may exert selective pressure, potentially leading to emergence of resistance against CSE itself. Thus, stewardship principles, judicious use, and local surveillance remain imperative [11].

Finally, limitations of this study must be acknowledged. The small sample size and single-center design restrict generalizability. Moreover, no molecular typing or resistance-gene profiling was conducted to confirm presence of ESBL or MBL genes; only phenotypic disk-diffusion testing was used. Minimum inhibitory concentration (MIC) testing was not performed, which could provide more nuanced understanding of susceptibility thresholds. There was no clinical correlation (i.e., no assessment of patient outcomes, therapeutic success, toxicity). Future studies should therefore include molecular characterization, MIC and PK/PD data, multicenter sampling, and ideally prospective clinical trials to validate the efficacy and safety of CSE in real-world settings.

CONCLUSION

The findings from this preliminary in-vitro evaluation suggest that the Ceftriaxone–Sulbactam–EDTA (CSE) combination exhibits strong antibacterial activity against a broad range of Gram-negative isolates from ICU clinical specimens, significantly outperforming ceftriaxone alone and a ceftriaxone–EDTA–Clavulanic acid regimen under the conditions tested. Given these results-together with supportive evidence from other Indian and international studies-CSE holds promise as a carbapenem-sparing therapeutic option, particularly in settings where ESBL and MBL producers are prevalent. Nonetheless, the current evidence remains insufficient for recommending widespread empirical use of CSE in clinical practice. Larger, multicenter studies incorporating molecular resistance profiling, MIC determination, PK/PD evaluation, and clinical outcome assessment are needed before CSE can be universally adopted as a standard therapy for MDR Gram-negative infections in ICUs.

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